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Chemotaxis of *Spirochaeta aurantia*: Involvement of Membrane Potential in Chemosensory Signal Transduction

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The effects of valinomycin and nigericin on sugar chemotaxis in *Spirochaeta aurantia* were investigated by using a quantitative capillary assay, and the fluorescent cation, 3,3'-dipropyl-2,2'-thiodicarbocyanine iodide was used as a probe to study effects of chemoattractants on membrane potential. Addition of a chemoattractant, D-xylose, to cells in either potassium or sodium phosphate buffer resulted in a transient membrane depolarization. In the presence of valinomycin, the membrane potential of cells in potassium phosphate buffer was reduced, and the transient membrane depolarization that resulted from the addition of D-xylose was eliminated. Although there was no detectable effect of valinomycin on motility, D-xylose taxis of cells in potassium phosphate buffer was completely inhibited by valinomycin. In sodium phosphate buffer, valinomycin had little effect on membrane potential or D-xylose taxis. Nigericin is known to dissipate the transmembrane pH gradient of *S. aurantia* in potassium phosphate buffer. This compound did not dissipate the membrane potential or the transient membrane depolarization observed upon addition of D-xylose to cells in either potassium or sodium phosphate buffer. Nigericin did not inhibit D-xylose taxis in either potassium or sodium phosphate buffer. This study indicates that the membrane potential but not the transmembrane pH gradient of *S. aurantia* is somehow involved in chemosensory signal transduction.

The chemotactic responses of bacteria are mediated by a number of discrete steps. Environmental stimuli interact with specific chemoreceptors directly. Information from the chemoreceptors is integrated, and this is followed by transduction of a signal to the organelle of motility. The motor function of the motility apparatus is then altered by the signal in such a way as to effect a modification of behavior (2, 3, 24, 32, 36, 37, 48).

Signal transduction is the least understood event among the steps leading to chemotaxis. It has been proposed that changes in membrane potential (54), intracellular Ca^{2+} concentration (47, 48) or cyclic GMP concentration (10) are involved in this process but experimental evidence is scarce and in some cases appears to conflict (42, 43, 54). The responses of a variety of eukaryotic sensory cells (e.g., neurons and protozoa) to chemoeffectors involve transient changes in membrane potential (4, 17, 33). Thus, it is of interest to ascertain whether membrane potential plays a role in the transduction of chemotactic signals in bacteria. It has been reported that a transient hyperpolarization of the membrane potential in *Escherichia coli* occurs in response to chemoattractants and chemorepellents and that this hyperpolarization is part of the chemotactic mechanism (54). Chemoeffectors have been reported to elicit a similar response in *Rhodopseudomonas sphaeroides* (5, 6). However, it was subsequently concluded that membrane potential was not involved in chemosensory transduction in *Bacillus subtilis* since changes in the membrane potential of this organism were not detected upon addition of chemoattractants (42). A recent investigation of the effects of a variety of attractants on the membrane potential and behavior of *E. coli* as well as *E. coli* chemotaxis mutants has led to the conclusion that behavioral changes induced by chemoeffectors are not mediated through membrane potential changes (M. A. Synder, J. B. Stock, and D. E. Koshland, Jr., J. Mol. Biol., in press).

Unfortunately, bacterial cells are too small to allow direct measurement of membrane potential with microelectrodes and indirect methods must be applied. These indirect methods have certain limitations and liabilities, for example each has a different sensitivity and different response time to changes in membrane potential. Thus, experiments such as those discussed above in which membrane potentials were monitored

by indirect means are open to a number of interpretations (42; A. Zaritsky, M. Kihara, and R. M. Macnab, J. Membrane Biol., in press). It would then be useful to employ a different approach to study the question of the relationship between membrane potential and bacterial chemotaxis as well as to continue efforts to determine the effects of chemoattractants on membrane potential. For this reason, we investigated the effects of a "voltage clamp" on sugar chemotaxis in *Spirochaeta aurantia*.

S. aurantia was chosen for several reasons. In a previous report, it was established that motility of *S. aurantia* was supported by a proton motive force (19) as is true of other bacteria (18, 29, 30, 34, 38–41, 52, 53). It was found that membrane potential could be monitored using a technique that employed the cationic, lipophilic carbocyanine dye, 3,3'-dipropyl-2,2'-thiodicarbocyanine iodide [Di-S-C_3(5)]. Furthermore, it was apparent that the potassium ionophore, valinomycin, would serve as a voltage clamp for *S. aurantia* under appropriate conditions. Even when the membrane potential had been dissipated with valinomycin, a membrane pH gradient served to support normal motility. Finally, a quantitative assay for chemotaxis in *S. aurantia* was described previously (20).

Spirochetes such as *S. aurantia* are characterized by a distinctive morphology. Generally, a spirochetal cell is helical and flexuous and possesses a protoplasmic cylinder which comprises the nuclear and cytoplasmic regions, as well as the membrane-peptidoglycan layer. Wrapped around the protoplasmic cylinder are structures called axial fibrils or periplasmic fibrils. One end of each periplasmic fibril is inserted near one pole of the protoplasmic cylinder, whereas the other end is not inserted. Since each periplasmic fibril is wrapped around most of the length of the protoplasmic cylinder, periplasmic fibrils inserted near one end of the protoplasmic cylinder overlap in the central region of the cell with periplasmic fibrils inserted near the opposite end. Both the periplasmic fibrils and the protoplasmic cylinder are enclosed by an outer envelope or outer membrane (11, 12, 15, 16). Periplasmic fibrils have been demonstrated to play a role in spirochetal motility (13, 49), and these organelles are similar in fine structure and chemical composition to bacterial flagella (8, 9, 16, 25–28, 35, 46). Although periplasmic fibrils are entirely endocellular organelles it is generally considered that the mechanisms of motility and chemotaxis in spirochetes are analogous to the mechanisms of motility and chemotaxis in flagellated bacteria (7, 19, 20).

The investigations reported herein were aimed at developing a better understanding of the mechanisms of chemotaxis in spirochetes. Furthermore, we felt that this information would be of use with respect to a general understanding of the processes by which signals are transduced from chemoreceptors to organelles of motility during bacterial chemotaxis.

MATERIALS AND METHODS

Bacterial strain and growth media. The organism used was *S. aurantia* M1 (12, 14). The culture medium contained 0.2 g of D-glucose, 0.5 g of Trypticase (BBL), and 0.2 g of yeast extract (Difco) per 99 ml of deionized water. After the pH was adjusted to 7.5 with KOH, the medium was autoclaved and then allowed to cool to room temperature. Finally, 0.5 ml of sterile 1 M potassium phosphate buffer (pH 7.0) was added to the medium. The medium used for colony counts consisted of the culture medium described above plus 0.75% agar (Difco).

Culture conditions. Cells for chemotaxis assays and Di-S-C_3(5) fluorescence measurements were grown in stationary tube cultures (20). Incubation was in air at 30°C, and cells were harvested from cultures in the late logarithmic phase (culture density, 1.5×10^8 cells per ml) by centrifugation (3,000 × *g* for 15 min at 4°C). The inocula (0.1 ml) for these cultures were from similar 5-ml cultures that had been incubated for 24 h.

Preparation of cell suspensions. Cells harvested from 5 ml of culture were washed once by suspending them in 5 ml of phosphate buffer (10 mM potassium or sodium phosphate as indicated plus 5 mM D-glucose and 200 μM L-cysteine hydrochloride). This is similar to the buffer used by Greenberg and Canale-Parola (20) to measure sugar chemotaxis in *S. aurantia*. After centrifugation, the washed cells were resuspended in buffer of the same composition as that in which they were washed, to a density of approximately 1.5×10^8 cells per ml, for use in studies of chemotaxis and membrane potential.

Chemotaxis assay. For measuring chemotaxis of *S. aurantia* a quantitative capillary assay similar to that described by Greenberg and Canale-Parola (20) was employed. This assay is based on the chemotaxis assay originally developed by Adler (1). Assays were carried out at 25°C for 20 min. Cells were suspended in either potassium or sodium phosphate buffer as indicated.

Detection of changes in $\Delta\Psi$. Previously described techniques (19, 39) that employ the cationic, lipophilic carbocyanine dye, Di-S-C_3(5), were used to monitor transmembrane electrical potential ($\Delta\Psi$) in *S. aurantia*. Di-S-C_3(5) was added to cell suspensions (final concentration, 0.5 μM), and fluorescence intensity at 670 nm was monitored with a Perkin-Elmer model 203 fluorescence spectrophotometer equipped with a strip chart recorder. Excitation was at 630 nm. The bacterial suspensions (2.5 ml) were continuously stirred within the cuvettes as measurements were made. Attractants and nonattractants were added to cell suspensions within the cuvette without interrupting fluorescence measurements. In all cases, the total volume added was 25 μl or less. Di-S-C_3(5) distributes across biological membranes in response to $\Delta\Psi$, intra-

cellular concentrations increasing as ΔΨ increases (cell interior negative), and uptake of this dye results in fluorescence quenching. Thus, as described previously (19), ΔΨ of *S. aurantia* was followed by monitoring Di-S-C_3(5) fluorescence. Calibration curves relating ΔΨ and fluorescence quenching were prepared by measuring quenching in cell suspensions containing valinomycin and potassium chloride at different concentrations, ΔΨ was calculated from the Nernst equation (39).

Chemicals. Sugars and valinomycin were purchased from Sigma Chemical Co. Di-S-C_3(5) was a gift from A. Waggoner, and sodium nigericin was a gift from R. Hamill. Valinomycin, nigericin, and Di-S-C_3(5) were dissolved in methanol. In cell suspensions containing these chemicals, the methanol concentration did not exceed 0.1%. This concentration of methanol did not affect ΔΨ or chemotaxis of *S. aurantia*. Aqueous solutions of other chemicals were used.

RESULTS

Chemotaxis of *S. aurantia* in the presence of valinomycin or nigericin. The source of energy for motility in *S. aurantia* is a proton motive force (Δp) which can be defined as the work required to move a proton from the outside of a cell to the inside (21, 22, 44, 45). The Δp consists of two components, the electrical potential between the inside and outside of a cell (ΔΨ) and the pH difference between the inside and outside of a cell (ΔpH), as indicated by the equation $\Delta p = \Delta\Psi - Z\Delta pH$, where Z is the factor used to convert ΔpH to millivolts (21, 22). When ΔΨ is dissipated a ΔpH can support motility of *S. aurantia* and vice versa (19).

Valinomycin conducts potassium ions across biological membranes (23, 50) and has been demonstrated to disrupt ΔΨ but not ΔpH in metabolizing cells of *S. aurantia* suspended in potassium phosphate buffer (19). Nigericin exchanges protons for potassium ions (23, 50) and has been shown to disrupt ΔpH but not ΔΨ in metabolizing cells of *S. aurantia* suspended in potassium phosphate buffer (19). Valinomycin and nigericin were used to test for involvement of ΔΨ and ΔpH, respectively, in chemotaxis of *S. aurantia*. The chemotactic response of cells suspended in potassium phosphate buffer (pH 7.0) towards D-xylose was inhibited by valinomycin, but nigericin appeared to effect a slight stimulation of this response (Fig. 1A). Similar results were obtained with other chemoattractants (D-glucose and D-mannose). These data suggest that ΔΨ but not ΔpH is involved in the response of *S. aurantia* to chemoattractants. Alternatively, valinomycin may block chemotaxis by a mechanism unrelated to interruption of ΔΨ. However, in sodium phosphate buffer, valinomycin did not inhibit D-xylose taxis (Fig. 1B). Since valinomycin should not affect ΔΨ in the absence of potassium, this experiment supports the notion that valinomycin inhibited chemotaxis in potassium phosphate buffer by interrupting ΔΨ.

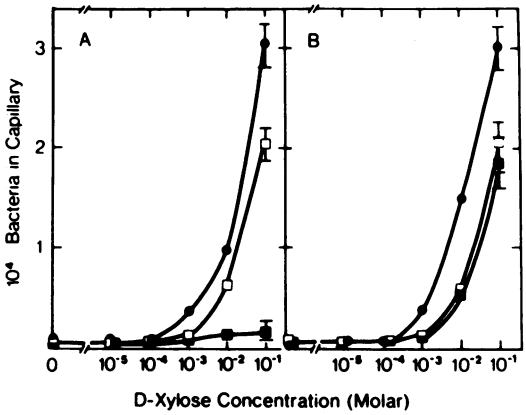

FIG. 1. *Concentration response curves for D-xylose taxis in the presence of valinomycin (25 nM) or nigericin (1 μM) as measured by using a quantitative capillary assay. (A) Potassium phosphate buffer (pH 7.0); (B) sodium phosphate buffer (pH 7.0). Nigericin in capillary and cell suspension (●), valinomycin in capillary and cell suspension (■), no inhibitors added (□). In potassium phosphate buffer, background accumulations of bacteria in capillaries without D-xylose were: 618 in the absence of inhibitors, 525 in presence of valinomycin, and 935 in presence of nigericin.*

As determined by plate counts, addition of valinomycin or nigericin to cells suspended in potassium phosphate buffer (incubation, 20 min at 25°C) did not result in a significant difference in viability as compared with a control which contained neither valinomycin nor nigericin. The presence of either antibiotic did not appear to alter the normal motile behavior of *S. aurantia* as described elsewhere (20), nor did these compounds significantly affect the outcome of quantitative capillary assays for motility (Fig. 1). When D-xylose was provided in both the capillary and the bacterial suspension at equal concentrations (10^{-1} M), accumulation of *S. aurantia* in the capillary was within the range observed in the absence of D-xylose (the background value). Thus, the response to D-xylose was a response to a concentration gradient of this attractant and not a consequence of stimulation of cell motility in the presence of D-xylose.

Effects of valinomycin and nigericin on ΔΨ. Once it had been established that valinomycin inhibited chemotaxis of cells suspended in potassium phosphate buffer, it was important to determine whether this agent actually served as a voltage clamp. Thus, studies of Di-S-C_3(5) fluorescence in cell suspensions were initiated (Fig. 2). Upon addition of 50 mM D-xylose to

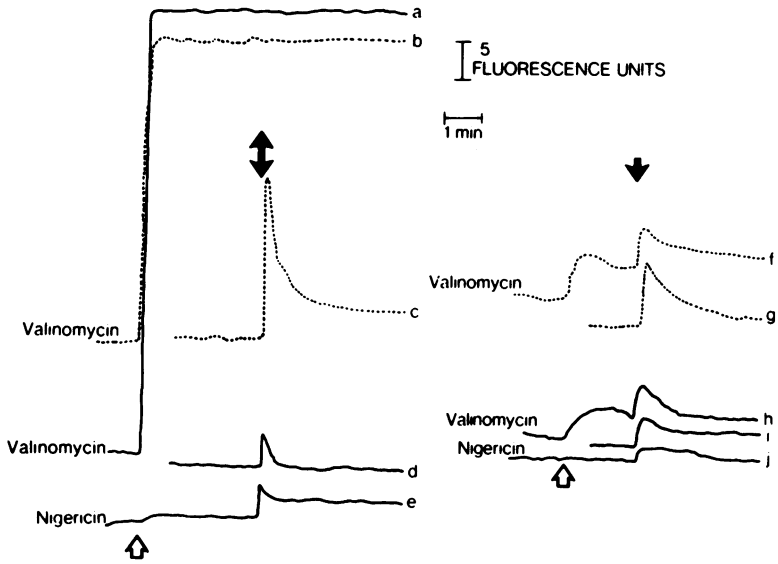

FIG. 2. *Fluorescence of Di-S-C_3(5) in suspensions of S. aurantia. Cells were suspended in potassium phosphate buffer (a to e) or in sodium phosphate buffer (f to j). Buffer, pH 7.0 (——); buffer, pH 5.5 (·····). Valinomycin (25 nM) or nigericin (1 μM) was added as indicated at the white arrow.* D-*Xylose (50 mM) was added at the black arrow.*

cells in potassium or sodium phosphate buffer (pH 7.0), a transient increase in fluorescence of Di-S-C_3(5) was observed (indicating a transient dissipation of $\Delta\Psi$). When valinomycin was added to cells in potassium phosphate buffer (pH 7.0), an increase in fluorescence that corresponded to a change in $\Delta\Psi$ from at least −90 mV (the maximum value that can be measured accurately with this technique [55]) to ca. −30 mV occurred. In the presence of valinomycin, addition of 50 mM D-xylose to cells in potassium phosphate buffer did not yield a fluctuation in Di-S-C_3(5) fluorescence. As expected, valinomycin exerted only a small effect on fluorescence of Di-S-C_3(5) when cells were suspended in sodium phosphate buffer, and the transient response observed upon addition of 50 mM D-xylose to cells in sodium phosphate buffer was not abolished by valinomycin. As indicated by Di-S-C_3(5) fluorescence, nigericin did not dissipate or clamp $\Delta\Psi$ of cells suspended in either sodium or potassium phosphate buffer.

Fluorescence of Di-S-C_3(5) becomes progressively less sensitive as $\Delta\Psi$ values exceed −90 mV. To optimize conditions for monitoring fluctuations in $\Delta\Psi$ with Di-S-C_3(5), we attempted to adjust $\Delta\Psi$ to a lower initial value than that obtained when cells were in either potassium or sodium phosphate buffer (pH 7.0). This was accomplished by suspending cells in pH 5.5 buffer. At pH 5.5, the ΔpH of *S. aurantia* is greater than at pH 7.0 (19), and although the $\Delta\Psi$ still exceeded −90 mV, it was less than at pH 7.0 as indicated by Di-S-C_3(5) fluorescence measurements (Fig. 2). Indeed, the magnitude of the transient change in Di-S-C_3(5) fluorescence that occurred upon addition of 50 mM D-xylose was greater at pH 5.5 than at pH 7.0 (Fig. 2). This may have been because of increased sensitivity of the fluorescent dye technique. Even at pH 5.5, valinomycin appeared to dissipate and clamp $\Delta\Psi$ of cells in potassium phosphate buffer, but had little effect on cells in sodium phosphate buffer.

Inhibition of chemotaxis at pH 5.5 by valinomycin. Since it appeared that the dye fluorescence technique for monitoring $\Delta\Psi$ was more sensitive at pH 5.5 than at pH 7.0, further studies on the effects of chemoattractants on $\Delta\Psi$ were carried out using pH 5.5 buffers. Thus it was important to demonstrate that *S. aurantia* exhibited a chemotactic response at pH 5.5 and that valinomycin inhibited this response in the presence of potassium. As shown in Fig. 3, cells suspended in either potassium or sodium phosphate buffer at pH 5.5 were attracted to D-xylose, although $\Delta\Psi$ was less at this pH than at pH 7.0 (Fig. 2). Valinomycin completely inhibited chemotaxis in potassium phosphate buffer but not in sodium phosphate buffer (Fig. 3).

Changes in $\Delta\Psi$ resulting from addition of chemoattractants. When a voltage clamp was applied to cells of *S. aurantia*, chemotaxis was

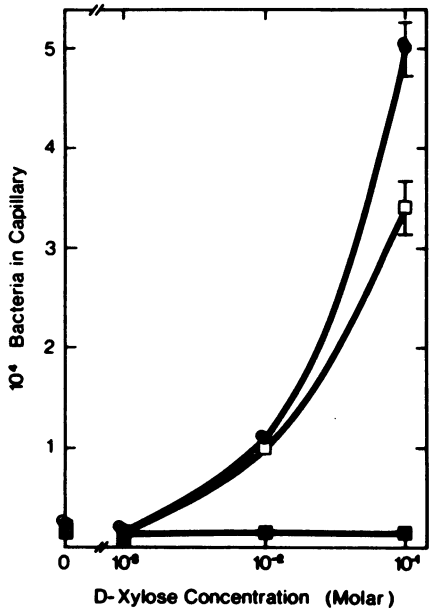

FIG. 3. *Concentration response curves for* D-*xylose taxis at pH 5.5. Potassium phosphate buffer* (●), *potassium phosphate buffer with 25 nM valinomycin present in capillary and cell suspension* (■), *sodium phosphate buffer* (○), *sodium phosphate buffer with 25 nM valinomycin present in capillary and cell suspension* (□). *In potassium phosphate buffer, background accumulations of bacteria in capillaries in the absence of* D-*xylose were 1,085 in the absence of valinomycin and 1,075 in the presence of valinomycin.*

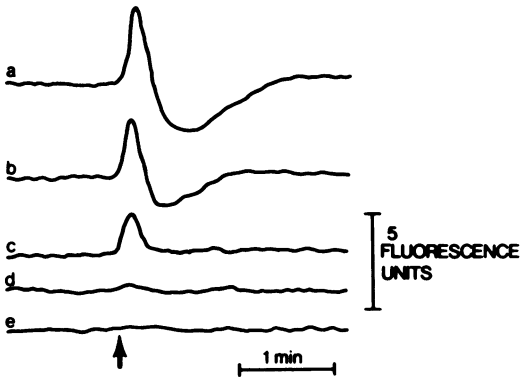

FIG. 4. *Fluorescence of Di-S-*C_3*(5) in suspensions of S. aurantia upon addition of various concentrations of* D-*xylose or addition of* D-*ribose. Arrow indicates addition of* D-*xylose at concentrations of: (a) 5 mM, (b) 1 mM, (c) 0.5 mM, (d) 0.1 mM, or (e) addition of 1 mM* D-*ribose to cells suspended in potassium phosphate buffer (pH 5.5).*

inhibited. This suggested that some sort of fluctuation in ΔΨ was a required part of the chemotactic mechanism in this spirochete. A range of D-xylose concentrations elicited a transient change in ΔΨ of cells suspended in potassium phosphate buffer, pH 5.5 (Fig. 4). Addition of 5 mM or 1 mM D-xylose caused a transient depolarization followed by a transient hyperpolarization as indicated by the Di-S-C_3(5) fluorescence signal. Fluorescence increased for 7 to 9 s, and then during the following 14 to 17 s, fluorescence decreased to a value below the original base line. After this apparent hyperpolarizing phase, the fluorescence signal gradually (~1 min) returned to the base-line value. Addition of 0.5 mM D-xylose resulted in a transient depolarization which was not followed by a hyperpolarizing phase. Addition of 0.1 mM D-xylose consistently caused a slight depolarization, however, the magnitude of this response did not exceed the magnitude of uninduced changes in the base line which were sometimes recorded. D-Ribose (1 mM), which is not an effective attractant for *S. aurantia* (20), did not affect Di-S-C_3(5) fluorescence (Fig. 4).

DISCUSSION

The experiments described in this paper indicate ΔΨ is involved in transduction of a chemosensory signal in *S. aurantia*. In the presence of potassium, valinomycin served to inhibit chemotaxis (Fig. 1 and 3). This treatment also served to partially dissipate ΔΨ in the metabolizing cells (to ca. −30 mV) and to clamp ΔΨ (Fig. 2). In the absence of potassium, valinomycin had little effect on ΔΨ and did not greatly affect chemotaxis (Fig. 1–3). Apparently, either the dissipation or the clamping of ΔΨ resulted in an inhibition of chemotaxis. At this time it is not clear which of these effects is involved in chemotaxis, although for several reasons, we favor the notion that it is the clamping effect. First of all, if it were the case that dissipation was inhibitory to chemotaxis because ΔΨ was an absolute requirement for a chemotactic response, it would have to be argued that the remaining level of ΔΨ under the conditions of the experiments was too low to meet this requirement even though it is a substantial value. Secondly, when cells were suspended in potassium phosphate buffer at pH 5.5, ΔΨ was decreased, as compared with cells at pH 7.0. The drop in external pH thus partially dissipated but did not clamp ΔΨ (Fig. 2). In the absence of valinomycin, the chemotactic response to D-xylose was somewhat greater at pH 5.5 than at pH 7.0 (Fig. 1 and 3). Although this provides evidence to suggest that clamping rather than decreasing ΔΨ inhibits chemotaxis, it should be pointed out that lowering the external pH did not result in as great a reduction of ΔΨ as did addition of valinomycin. Perhaps the magnitude of the reduction was not large enough to inhibit

chemotaxis.

Nigericin disrupts ΔpH of *S. aurantia* cells in the presence of potassium (19) but had little effect on ΔΨ in the presence or absence of potassium (Fig. 2). Chemotaxis was not inhibited by nigericin (at pH 7.0); in fact, nigericin stimulated chemotaxis slightly in the presence or absence of potassium (Fig. 1). Since nigericin did not inhibit chemotaxis in the presence of potassium it appears that ΔpH is not a chemosensory signal during sugar chemotaxis in *S. aurantia*. It has been reported that the behavior of the flagellated bacteria, *Salmonella* sp., *B. subtilis*, and *E. coli* can be altered by changes in the proton motive force (29, 42, 43). Although it does not appear that ΔpH is involved in chemosensory transduction of *S. aurantia*, it is possible that the ΔΨ involvement is related to a mechanism which can sense changes in Δp.

In potassium phosphate buffer at pH 5.5 plus nigericin, cells did not exhibit coordinated motility. Rather, they appeared to flex incessantly (data not shown). The reasons for this are unclear. One possibility is that the cells become acidified and that this disrupts normal motile behavior. This interpretation is supported by recent demonstrations that nigericin increases the frequency of tumbling in *E. coli* and *Salmonella* sp. and that this behavior modification is due to a decrease in the intracellular pH (31, 51).

The membrane potential of *S. aurantia* was monitored indirectly by means of the fluorescent probe, Di-S-C_3(5). When attractants were added to cells, a transient depolarization was observed. Addition of D-xylose to a final concentration of 5 or 1 mM resulted in a transient depolarization followed by a transient hyperpolarization before the fluorescence signal returned to the base line (Fig. 4). At lower concentrations (Fig. 4) of D-xylose and at the highest concentration tested, 50 mM (Fig. 2), only a transient depolarization was observed. Addition of sugars that do not serve as effective attractants for *S. aurantia* (e.g., D-ribose) did not induce detectable fluctuations in the fluorescence signal from Di-S-C_3(5). These data are consistent with the hypothesis that the transient changes in membrane potential resulting from addition of attractants are involved in the chemotactic response of *S. aurantia*. However, the present evidence is not conclusive, and further investigations are required to clarify this point. For example, we cannot rule out the idea that the energy required for transport is related to the fluctuations in ΔΨ which were observed upon addition of effective chemoattractants. To test this hypothesis, chemotaxis mutants and transport mutants should be isolated, and the responses of their ΔΨ to chemoattractants should be determined.

Szmelcman and Adler (54) reported that a transient hyperpolarization in membrane potential was part of the chemotactic mechanism of *E. coli*. A technique involving equilibrium distribution of the permeant cation, triphenylmethylphosphonium, was employed to monitor membrane potential. Subsequently, Miller and Koshland (42) reported that the chemoattractant L-alanine did not elicit a detectable change in the membrane potential of *B. subtilis* as monitored by a Di-S-C_3(5) technique similar to that described herein. We now report that a transient depolarization of membrane potential results from addition of chemoattractants to *S. aurantia* (Fig. 4). It may be that each of these diverse bacterial species uses a different system for chemosensory transduction; however, particularly in the case of *E. coli* and *B. subtilis*, there is evidence to indicate that this is not the case (42). Although we have demonstrated certain similarities between the motility and chemotaxis systems of *S. aurantia* and flagellated bacteria (19, 20), it remains a distinct possibility that there are basic differences in the mechanisms of chemosensory transduction of spirochetes and the flagellated bacteria which have been studied. In fact, it has been argued that low-molecular-weight compounds could diffuse across the length of a cell as small as an *E. coli* cell in a short enough time to make chemical transmission of chemosensory information feasible. However, in longer cells such as *S. aurantia*, chemical signals would be too slow, and it might be expected that an electrical signal would be involved in regulation of swimming behavior (Snyder et al., in press). Another explanation for the observed differences in the response of ΔΨ to chemoattractants in the three different bacterial groups discussed may relate to the problems inherent in the available techniques for monitoring ΔΨ in actively metabolizing bacteria. For example, the response of ΔΨ upon addition of chemoattractants to *S. aurantia* cells as measured by the Di-S-C_3(5) technique depended on the energy source provided in the suspending buffer. When D-xylose was used in place of D-glucose as an energy source, the basal ΔΨ value was lower. Although addition of 10 mM D-glucose to cells suspended in 5 mM D-glucose resulted in a transient depolarization such as those described above (Fig. 4), addition of 10 mM D-glucose to cells suspended in 5 mM D-xylose resulted in a permanent hyperpolarization (unpublished data). These data can be interpreted as a masking of the depolarizing effect of D-glucose by an increased metabolism leading to an increased ΔΨ when D-glucose is added to cells with D-xylose as the energy source.

Obviously, interpretations of results of studies aimed at determining the effects of chemoattractants on $\Delta\Psi$ are complicated. However, now that evidence for involvement of $\Delta\Psi$ (but not ΔpH) in chemosensory transduction of *S. aurantia* has been provided by means of the voltage clamp experiments described herein, the effects of chemoattractants on $\Delta\Psi$ should be studied more rigorously.

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